PATENT
3645-0104P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: Richard B. THOMPSON et al. Conf.: 9931

Appl. No.: 09/942,708 Group:

Filed: August 31, 2001 Examiner:

For: DETERMINATION OF METAL IONS IN SOLUTION BY PHOTOLUMINESCENCE ANISOTROPY

LETTER TO THE OFFICIAL DRAFTSPERSON

Assistant Commissioner for Patents
Washington, DC 20231

December 11, 2001

Sir:

Attached hereto is/are one (1) sheet(s) of formal drawings which comply with the provisions of 37 C.F.R. § 1.84. The drawings should be made a part of the record of the above-identified application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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By _____
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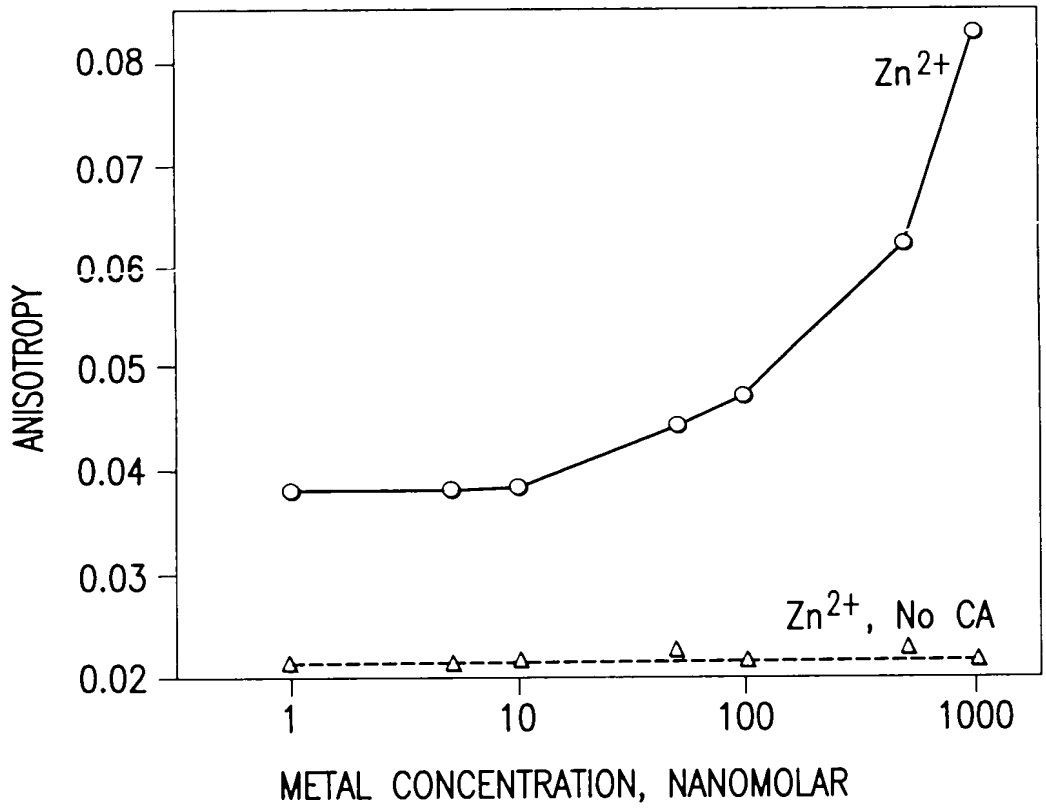
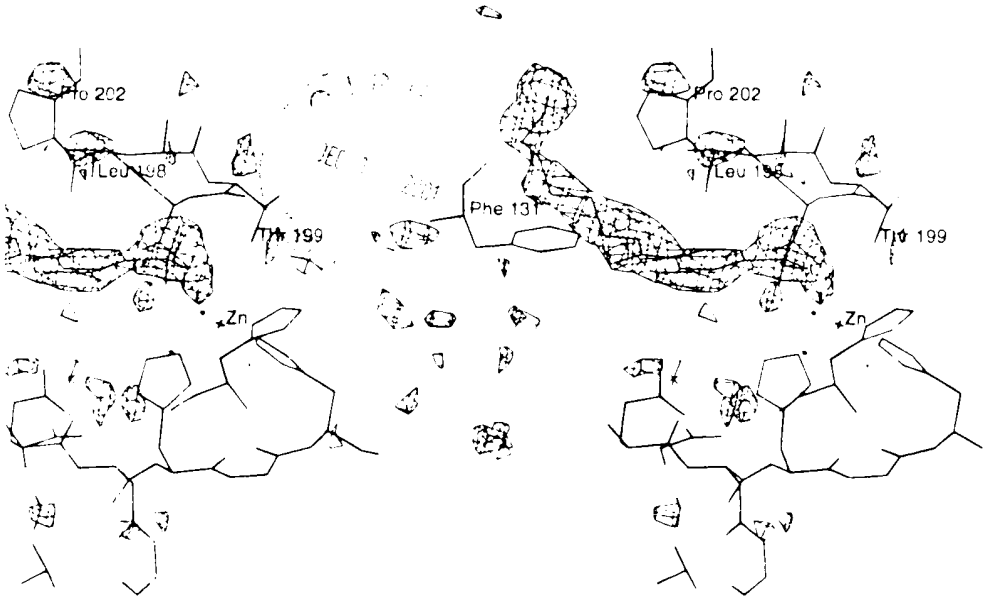
DRN/crt
3645-0104P

Attachments

Figure 9


0.08
0.07
0.06
0.05
0.04
0.03
0.02
ANISOTROPY
Zn 2+
Zn 2+, No CA
1
10
100
1000
METAL CONCENTRATION, NANOMOLAR



CAII−**3** complex contoured at 2.4 σ. Enzyme residues Phe-131, Leu-198, Thr-199, Pro-202, and Zn^{2+} ut to the thiocarbonyl moiety. No electron density is observed for the fluorescein portion of compound

ns

separation (Å)	
2	**3**
2.1	2.4
2.8[a]	3.1[a]
3.1[a]	3.5
3.3	3.3
3.3	3.6
2.9[a]	
3.3	2.9
3.0[a]	

dged from distance and

ond contacts with the
s. Thus, only hydro-
202 appear to stabilize
tail of **2**. The protein
on binding **2**. This is
4-((glycyltriethylene
extensive hydrophobic
98 region stabilize the
r in a nearly identical

d above, there are two
n CAII and **2**. The
ond from water 401,
from the side chain
ide of the active site
en bond to water 302.

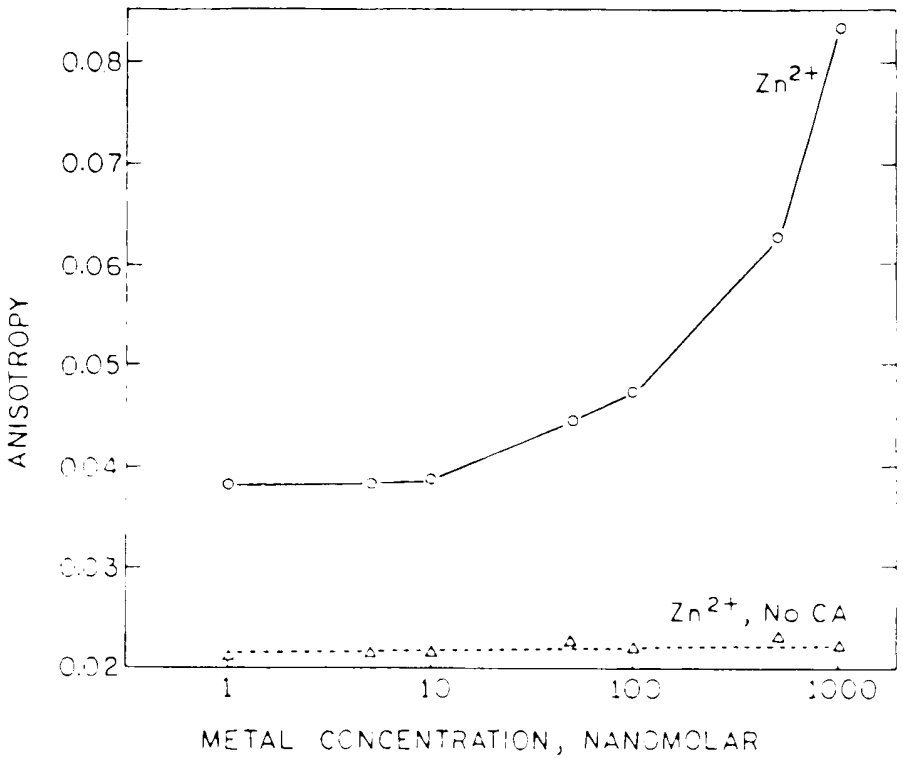


Figure 5. Zn^{2+} concentration-dependent fluorescence anisotropies of 1 μM **3** are depicted in the absence (triangles) and presence (circles) of 1 μM apo-CAII. Anisotropy = $(I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$; see eq 6.

An electron density map of the CAII−**3** complex is shown in Figure 4, and selected enzyme−inhibitor interactions are recorded in Table 2. We note that the hydrophobic and hydrogen bond interactions described in the previous paragraph are presumably sufficient to stabilize inhibitor binding to the apoenzyme, although with ~10^3-fold weaker affinity, in the absence of sulfonamide–zinc coordination.